simulations implemented in Rosetta to probe the heterogeneous, dynamic and transient ensemble of IAPP oligomers and define structural models for a family of membrane-bound IAPP dimers [Nath, Miranker & Rhoades. (2011) Angew. Chemie. Intl. Ed. 50(46):10859]. Here we describe efforts, based on this novel structural insight, to design and identify small molecules and peptides that bind selectively to particular configurations of IAPP dimers, and thereby bias the oligomeric ensemble so as to alter membrane-binding and subsequent toxicity mediated by higher-order states. Small molecules were identified from large commercial libraries ($N > 100000$) using a virtual screening protocol incorporating computational docking and statistical inference, while short, selective peptides were generated using RosettaDesign. The effects of these compounds on IAPP membrane-binding, conformation and cytotoxicity were then characterized using fluorescence correlation spectroscopy (FCS), spFRET and cell viability assays. Active modulators could serve as valuable tools in mechanistic studies of membrane disruption mediated by IAPP, and might also function as leads for new therapeutics targeting T2DM. In addition, the strategies developed here could serve as a framework for efforts targeting other clinically-relevant amyloidogenic intrinsically disordered proteins such as α-synuclein, amyloid-β and tau.

291-Pos Board B60

Effect of Prolines on the Intrinsic Stiffness of Islet Amyloid Polypeptide Variants

Stephanie M. Cope, Sara M. Sizemore, Anindya Roy, Giovanna Ghirlanda, Sara M. Vaiana.
Arizona State University, Tempe, AZ, USA.

Human islet amyloid polypeptide (hIAPP) is an intrinsically disordered hormone that is co-secreted with insulin in the beta-cells of the pancreas. The aggregation of hIAPP into insoluble amyloid fibrils is believed to play a causal role in type 2 diabetes. The rodent variant (rIAPP), differing by 6 of 37 amino acids, does not aggregate into amyloid fibrils and inhibits hIAPP amyloid formation. Both these properties have been attributed to rIAPP's three proline mutations (A25P, S28P and S29P). Single proline mutants of hIAPP have also been shown to kinetically inhibit hIAPP fibril formation. Because of their intrinsic dihedral angle preferences, prolines are expected to affect monomer conformational sampling. However, the specific effect of proline substitutions on IAPP structure and dynamics has not yet been explored. Detecting such properties is challenging due to the low molecular weight, fast reconfiguration times, and very low solubility of IAPP peptides. High resolution, time resolved techniques are needed.

We use a nanosecond laser spectroscopy technique to measure end-to-end contact formation rates in IAPP mutants. Using this technique, Vaiana et al. showed that rIAPP populates more extended conformations, characterized by larger end-to-end distances, compared to hIAPP. Here we study the effects of proline substitutions in IAPP and characterize them in terms of intrinsic chain stiffness. We find that the three proline mutations alone do not explain rIAPP's increased chain stiffness. Interestingly, early experiments by Green et al. show that mutating the non-proline residues in rIAPP renders it capable of forming amyloids. Together these results suggest that rIAPP's unique chain stiffness is a determinant for its non-amyloidogenic properties. We discuss the reasons for this peptide's unique chain stiffness and the implications of our findings on the effect of prolines in IDPs.

Protein Dynamics I

292-Pos Board B61

Path Searching Towards the Symmetric Inward Open Structure of LeuT

Michelle A. Sahai[1], Benjamin Burnett[1], Sebastian Stolzenberg[1], Lei Shi[1], Harel Weinstein[1,2].
[1]Department of Physiology and Biophysics, Weill Cornell Medical College of Cornell University, New York, NY, USA, [2]HRH Prince Alwaleed Bin Talal Bin Abdulaziz Alsaud Institute for Computational Biomedicine, Weill Cornell Medical College of Cornell University, New York, NY, USA.

Few crystal structures are available for transporters related to the biomedically important neurotransmitter transporters, such as the serotonin and dopamine transporters. One of these structures is the bacterial analog LeuT, which transports Leucine. Until recently the available crystal structures of LeuT provided information on only two distinct states: an outward open state, where the S1 substrate-binding site is exposed to the extracellular vestibule, and an occluded state, where the S1 binding site is occluded from both the extracellular and intracellular vestibules. Therefore, there was not enough structural information to support an understanding of the substrate transport mechanism. Several computational models emerged to describe the inward facing conformation of LeuT in which the substrate is released. Given the structural information, some of these models (e.g., the symmetric inward open model - SIO) assumed rigid body motions in which the molecule would transition between the outward open-occluded-inward open states. We attempt to evaluate the feasibility of various models of LeuT by generating a transition path between distinct conformational states using the Motion Planning (MP) module Pathrover, a method that can identify a set of low-energy, clash-free structural intermediates between known end states. Because the sodium-hydantoin transporter Mhp1, which transitions between the same kind of states, has been crystallized in three distinct conformations, and intermediates have been calculated from a force-based approach, dynamic importance sampling (DIMS), it was used here to test the robustness of the Pathrover approach. For Mhp1 we find a clear overlap between the Pathrover computed intermediates and the DIMS intermediates, and conclude that the transition among the Mhp1 states is well represented by Pathrover. The intermediates calculated for LeuT in this work form the basis for comprehensive molecular dynamics simulations probing the molecular mechanism.

293-Pos Board B62

Molecular Modeling and Molecular Dynamics Simulations of Recombinase Rad51

Yuichi Kokabu, Mitsunori Ikeguchi.
Yokohama City University, Yokohama, Japan.

DNA strand exchange is the central reaction of homologous recombination, which is catalyzed by Rad51 recombinase in eukaryotes. In the reaction, a filament comprised of Rad51 and ssDNA searches a homologous DNA strand, and exchanges those strands. In the presence of ATP, Rad51 can form active filaments.

The yeast Rad51 dimer structure in the active form of the filament was constructed using homology-modeling techniques, and all-atom molecular dynamics (MD) simulations were performed using the modeled structure. We found two crucial interaction networks involving ATP: one is among the γ phosphate of ATP and K^+ ions; the other is among the adenine ring of ATP.

Multiple MD simulations were performed in which the number of bound K^+ ions was changed. The simulated structures suggested that K^+ ions are indispensable for the stabilization of the active dimer and resemble the arginine and lysine fingers of other P-loop containing ATPases and GTPases.

Furthermore, in MD simulations starting from a structure just after ATP hydrolysis, the opening motion corresponding to dissociation from DNA was observed. These results support the hypothesis that ATP functions as ''glue'' between protomers.

294-Pos Board B63

A Structural and Dynamics Survey of HIV-1 Reverse Transcriptase

James M. Seckler, Hongyu Miao, Alan M. Grossfield.
University of Rochester, Rochester, NY, USA.

HIV-1 reverse transcriptase is a critical drug target for HIV treatment, and understanding the exact mechanisms of its function and inhibition would significantly accelerate the development of new anti-HIV drugs. RT is a heterodimeric, multifunctional, multidomain protein with a 66 kDa subunit containing all of the catalytic active sites, and a 51 kDa subunit which is thought to provide structural stability to the larger subunit. Structural information on reverse transcriptase alone has proven to be insufficient to explain the mechanism of inhibition and drug resistance of non-nucleoside reverse transcriptase inhibitors. Elastic network modeling provides a technique to rapidly probe and compare protein dynamics. Combining elastic network modeling with hierarchical clusters of both structural and dynamic data reveals a wealth of novel information. Here we present an extensive survey of the dynamics of reverse transcriptase bound to a variety of ligands with a number of mutations, revealing a novel mechanism for drug resistance to non-nucleoside reverse transcriptase inhibitors, where hydrophobic core mutations subtly shift the position of the thumb subdomain, restoring active-state motion to multiple functionally significant regions of HIV-1 RT. This model arises out of a combination of structural and dynamic information, rather than exclusively from one or the other.

295-Pos Board B64

Computational Studies of Binding of Small Molecule Inhibitors to the DNA Binding Protein Menin

Joe Jordan, Shenghao Jin, Xianxin Hua, Ravi Radhakrishnan.
The University of Pennsylvania, Philadelphia, PA, USA.

The DNA-binding protein menin is known to play important roles in a number of cancers and may have a role in type-II diabetes (T2D). In particular,